

Comparative Activities of Ethanol and Metabolic Extracts Of aloe Barbadensis Millerton Clinical Isolates Of candida Albia's

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Abstract:

Background: *Aloe barbadensis miller* (Aloevera) is a known medicinal plant belonging to the Liliaceae family. The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on their upper and lower stem surfaces. The slimy gel in the aloevera leaf has traditionally been used for treatment of digestive tract disturbances, sunburn and wounds. The therapeutic claims for aloevera cover a broad range of conditions. It is commonly used topically in the treatment of dermatological conditions such as dermatitis. An evaluation study to determine the antifungal activities of methanolic and ethanolic extracts of aloevera on *Candida albicans* isolates was carried out.

Method: The extracts from the aloevera plant was tested for antifungal activity via invitro study at various concentrations using the agar well diffusion method. A standard antifungal, fluconazole was used as positive control. Dimethylsulfoxide (DMSO) was used as the diluting solvent of the extract and also as negative control. Minimum inhibitory concentration (MIC) values of the extracts and antifungal agent on the fungal isolates that were sensitive to them were also determined using concentrations ranging from 3.12mg/ml to 50mg/ml by agar dilution method. The concentration ranges of fluconazole used was 0.125µg/ml to 64µg/ml.

Results: From the sensitivity performed, both ethanol and methanol extracts showed antifungal activity against the fungal isolate at 50mg/ml. Ethanol extract showed wider zones of inhibition. The MIC values of methanol extract varied between 12.5mg/ml to 25mg/ml while MIC of ethanol extract varied between 6.25mg/ml and 12.5mg/ml. The MIC of fluconazole varied between 4µg/ml and 16µg/ml.

Conclusion: Considering the relatively low MICs recorded in this study, it can be said that the organic leaf extract of *Aloe barbadensis miller* could be useful as an alternative to orthodox antifungals in the treatment of infections caused by *Candida albicans*.

Key words: *Aloevera*, antifungal activity, *Candida albicans*

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I. Introduction

Aloe barbadensis miller, commonly called Aloevera grows in the wild in tropical climates around the world and is used for medicinal purposes. Aloevera is also used for decorative purposes and grows successfully indoors as a potted plant (Perkins, 2016). Like other aloe species, aloevera forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients. More than 75 active agents have been attributed to Aloevera. The gel consists of 98 – 99% water and the remaining 1 – 2% contains the active compounds such as acetylated mannans, sterols, amino acids, vitamins, polymannans, anthra-quinone, C-glycosides, anthrones and other anthra-quinones such as emodin and various lectins (King *et al.*, 1995; Hutter *et al.*, 1996; Eshun and He, 2004; Boudreau *et al.*, 2006).

Aloevera has long been associated with the treatment of burns. Aloevera ointment can be used for healing burns on the skin (Perkins, 2016). It has been used in the treatment of a wide range of conditions such as alopecia, Alzheimer's disease, congenital heart failure, depression, glaucoma, hemorrhoids, hepatitis, varicose veins, metastatic cancer, ulcers, Diabetes mellitus and inflammation of the gastrointestinal tract (Coronado *et al.*, 2004). Aloevera latex is commonly used in the treatment of constipation (Dewitte, 1993).

Candida albicans is a dimorphic fungus that grows both as yeast and filamentous cells and one of the few species of the genus *Candida* that causes the infection candidiasis in humans (Martins *et al.*, 2014; Erdogan *et al.*, 2015). *Candida albicans* is responsible for 50 – 90% of all cases of candidiasis in humans (Martins *et al.*, 2014). Candidiasis is often observed in immune-compromised individuals, including HIV-

infected patients. It commonly occurs on mucous membranes in the mouth or vagina but may affect a number of other regions. In addition, an overgrowth infection is considered superinfection, usually applied when an infection becomes opportunistic and very resistant to antifungals. It may then become suppressed by antibiotics. The infection is prolonged when the original sensitive strain is replaced by the antibiotic resistant strain (Turano *et al.*, 2003). This study was carried out to investigate the antifungal activity of ethanolic and methanolic extracts of aloe vera on *Candida albicans*.

II. Materials and Methods

Clinical isolates of *Candida albicans* were obtained from the Microbiology laboratory, University of Calabar Teaching Hospital and used for this study. The aloe vera plant was obtained from the University of Calabar Botanical Garden.

The aloe vera leaves were properly washed with distilled water and chopped into small sizes. The leaves were then soaked in 1000ml of each of ethanol and methanol. The mixtures were allowed to stand at room temperature for 72 hours to enable the active organic components to dissolve in the extracting solvents. After filtration with sterile Whatman No. 1 filter paper, the extracts were evaporated to dryness with the use of a water bath (F. – Nr.; L5008.0271 type) carefully monitored at 45°C to give a crude pasty residue which was weighed (12g for ethanol and 11g for methanol). The pasty residues were then placed in sterile universal bottles and preserved at 4°C until required. Each step was done aseptically.

Candida albicans produced creamy to whitish tiny pasty colonies on Sabouraud dextrose agar (SDA). The germ tube test was performed to properly identify isolates of *Candida albicans*. The sensitivity testing of the crude plant extracts was determined using agar-well diffusion method. A sterile cotton-tipped swab was dipped into the standardized inoculum suspension and used to streak Sabouraud dextrose agar. This was allowed to dry prior to boring four wells of 6mm diameter each. The wells were then labeled “M” for methanol extract, “E” for ethanol extract, “F” for fluconazole (standard antifungal) and “D” for dimethylsulfoxide (DMSO). Each well was filled accordingly with a drop of crude extract at 50mg/ml and fluconazole at 64 µg/ml and DMSO. Each plate was allowed to stand for about 30 minutes for diffusion to take place before being inverted and incubated aerobically at 37°C for 24 hours. Growth inhibition zone diameters (mm) were measured using calibrated transparent ruler and the results were recorded. Positive and negative controls were maintained with fluconazole and DMSO respectively. Five isolates of *Candida albicans* were used.

The plant extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare different concentrations ranging between 3.125mg/ml and 50mg/ml. For fluconazole 0.125µg/ml to 64µg/ml concentration ranges were prepared using sterile distilled water.

III. Results

The results for antifungal screening test (zone of inhibition (mm) for the extracts from *Aloe babadensismiller* at 50mg/ml and standard antifungal fluconazole at 64µg/ml and DMSO (solvent) against 5 isolates of *Candida albicans* are shown in table 1. Both methanol and ethanol extracts of the plant showed broad spectrum antifungal activity against all the isolates. Fluconazole also showed activity against all the isolates. The solvent (DMSO) used for the dissolution of the extract, showed no activity. The MIC value of methanol extract varied between 12.5mg/ml and 25mg/ml while MIC of ethanol extract varied between 6.25mg/ml and 12.5mg/ml. The MIC value of fluconazole varied between 4µg/ml and 16µg/ml for the different isolates.

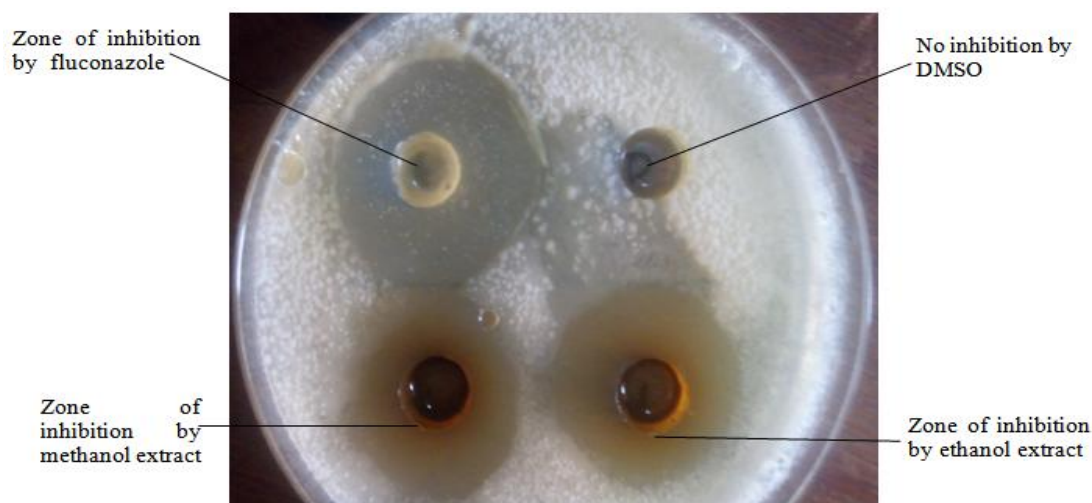


Plate 1: Antifungal activity of *Aloe barbadensismiller* extracts and fluconazole on *Candida albicans* isolate

Table 4.1: Antifungal activity of extracts of *Aloe barbadensis miller* against *Candida albicans*

Isolate	Methanol extract			Ethanol extract			Fluconazole		
	NIS	MDZI (mm)	NINS	NIS	MDZI (mm)	NINS	NIS	MDZI (mm)	NINS
<i>Candida albicans</i>	5	14.60	0	5	16.60	0	5	21.00	0

Table 4.2: Minimum inhibitory concentration (MIC) of methanol and ethanol extracts of *Aloe barbadensis miller*

Isolate	Methanol extract (mg/ml)	Ethanol extract mg/ml	Fluconazole (mg/ml)
<i>Candida albicans</i>	12.5	6.25	16

IV. Discussion

The increasing resistance of fungal organisms to common antifungal agents has become a major public health concern. This has led to the development of antifungals from natural products to control fungal diseases. Obviously natural plant products will continue to be important as sources of medicinal agents as they have been used traditionally for thousands of years by many cultures for controlling common health issues (Richardson, 1993; Aboaba, 2001).

Due to the occurrence of re-infection by multi-drug resistant strains, natural plant based drugs have been accepted and widely used for treatment of infections. The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids. It is interesting to note that even crude extract of these plants showed good activity against multi-drug resistant strains where modern antifungal therapy had limited effect (Surjusheet *et al.*, 2008).

The antifungal activity and inhibitory activity of aloe vera extract could be attributed to the chemical properties of aloe vera. The gel contains an emollient polysaccharide, glucomannan (Dagneet *et al.*, 2000; Ahirwar and Jain, 2011). It is a good moisturizer which accounts for its use in many cosmetics. Acemannan, the major carbohydrate fraction in the gel, is a water soluble long chain mannose polymer which accelerates wound healing, modulates immune function and demonstrates antineoplastic and antiviral effects (Penget *et al.*, 1991). The leaf lining (latex, resin, or sap) contains anthraquinone glycosides (aloin, aloe-emodin and barbaloin) that are potent stimulant laxatives. The gel also contains bradykininase (Yagiet *et al.*, 1982; Choi and Chung, 2003). Aloe vera contains six antiseptic agents; Lupeol, salicylic acid, urea, nitrogen, cinnamonic acid, phenols and sulphur (Ahirwar and Jain, 2011). They all have inhibitory action on fungi, bacteria and viruses (Atherton, 1998; Surjusheet *et al.*, 2008). It has been shown that when solvents like methanol, ethanol, hexane and acetone are used to extract plant components, most of them are able to exhibit inhibitory effect on fungi and gram positive and gram negative bacteria (Bushra and Ganga, 2003).

The results of this study suggest that ethanol and methanol extracts of aloe vera may be used for the treatment of fungal infections due to some resistant fungi such as *Candida albicans* (Martins *et al.*, 2014). The richness of aloe vera with vitamin C, E and beta carotene gives it its nourishing and anti-ageing qualities, hence, the plant extract may be used to treat wounds, minor cuts, dry skin and severe burns. Aloe vera can improve the effectiveness of one's diet and also maximize weight loss potential (Zadiket *et al.*, 2010).

Therefore, alternative medicine should be fully recognized by all levels of government and policy makers for its contribution to health care, as this would open up opportunities for collaboration between alternative and orthodox medicine in combating the increasing resistance to antifungals and antimicrobials in general.

V. Conclusion

This study has shown that aloe vera crude extracts possess antifungal activity and that the inhibition of fungal growth was dose dependent. The results suggest that ethanol and methanol extracts of aloe vera may be used for the treatment of fungal infections due to some drug resistant fungi such as *Candida albicans*. The active component of this medicinal plant should be extracted, purified and formulated for topical application and oral ingestion in order to make it more appealing and user-friendly.

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